CYTOLOGICAL DEMONSTRATION OF THE HELICAL STRUCTURE OF GIANT CHROMOSOMES

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Alverdes[1] as long ago as 1912 interpreted the bands of the giant chromosomes found by Balbiani[2] in *Chironomus* as the result of spiralization. Later, Kaufmann,[3] applying plant-chromosome techniques to *Drosophila* giant chromosomes, concluded that the discoid or discontinuous appearance of these chromosomes was an expression of inadequate technique and that a spiral structure was the more accurate. Nevertheless, the discontinuous structure appeared to fit genetic data more easily.

Evidence from embryology[4, 5] indicates that the bands or "disks" of the giant chromosomes of the Diptera have their origin in the turns of a helical (spiral) structure. In view of the difficulty of discerning connections between the bands, however, this evidence lacked the necessary proof that the fully formed chromosomes were themselves spiral; in fact, it had been postulated that the coils actually broke to form rings. Evidence of a cytological nature has also been given[6] that the bands of the giant chromosomes are composed of the turns of many small helices which together appear to be a band. These data have been marginal in optical resolution, however. Other evidence[7] of a cytological nature depends largely upon a characteristic resolution of the microscope which is of uncertain meaning. Serial sections of *Drosophila* chromosomes[8] studied with the electron microscope appear to indicate that the structure is that of a hierarchy of helices, which appears to be similar to that found with isolated chromosomes.[9]

In the course of studying *Drosophila* giant chromosomes for the purpose of attempting to detect connections between the bands, a portion of one chromosome was found which showed a considerable area in which the bands actually were connected as in a helix. Further study has revealed that these connections are not uncommon, but it is rare to find one as extensive as this. To illustrate the helical nature, the strand was photographed from top to bottom at different focal levels by changing the fine focus adjustment of the microscope. The series of prints obtained does indeed show that the bands of the chromosome are in reality turns of helices and that the bands of the diploid or paired chromosomes are in fact composed of regularly arranged turns of two helices.

The series of prints is shown in Figure 1. Starting with one focal level at one end and proceeding to the other end, the turns of the helices can be followed all around the circumference of the coils between points *a* and *b* on the chromosome. In prints *A–F* the strands are slanted in the reverse direction from those in prints *J–K*. The central section of the helix, prints *G–I*, shows portions in both directions, as would be the case were one actually looking at a true helix.

The groove separating the paired homologues of the diploid chromosome is seen slightly below the middle. At *H* the spiral of one half of the chromosome is indicated to be independent of the spiral of the other half. In Figure 2 the same chromosome is shown flattened further by compression between the slide and coverslip. Being in a flatter field, the strands are seen over most of their distance

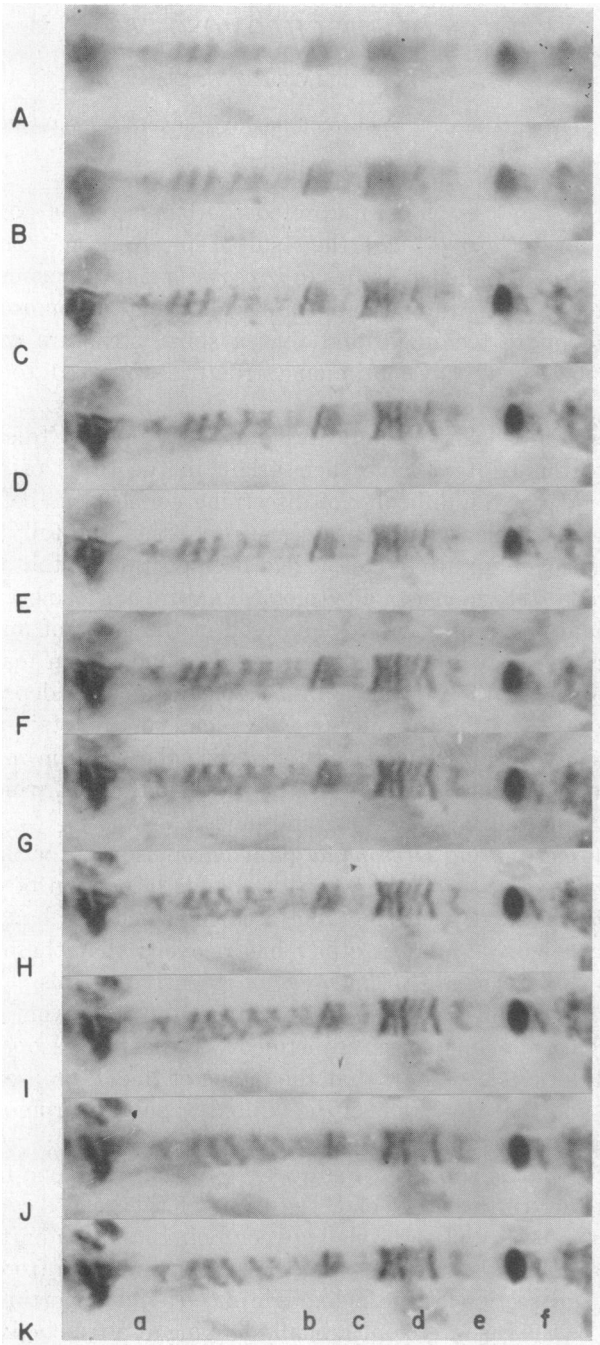

FIG. 1.—Part of a chromosome of *Drosophila melanogaster* (stained and fixed with aceto-orcein and smeared on a slide), photographed at successive focal-plane levels from top to bottom. Oil immersion obj. N.A. 1.32.

as they traverse a helical course and thus are seen to be proceeding in both directions at the same time. A model showing two helical wires representing the paired homologues is shown in Figure 3. Owing to the great depth of focus of the camera lens, all portions of the wire model are in focus at the same time, the lower parts having been shaded so as to indicate depth. However, in Figure 3, *B*, the upper portions of each coil are darkened; the appearance then is that of the chromosome pair in Figure 1, *A–F*. In Figure 3, *C*, the lower part of each coil is darkened and the appearance is that of the chromosome in Figure 1, *J–K*.

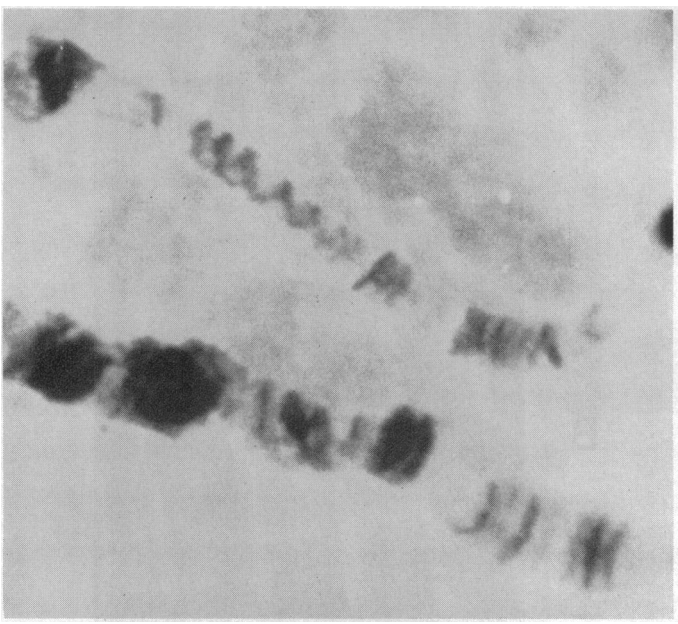

FIG. 2.—Same chromosome as in Figure 1 after a greater pressure on the coverslip. Note the further stretching between the bands.

From this, the larger bands of the diploid giant chromosomes are shown to be composed of the combined coils of the synapsed haploid strands. Furthermore, the bands are continuous from band to band, being connected together as a double helical structure, with the coils symmetrically arranged so that the "bands" appear to be paired. The close compression of the coils in the usual preparation makes it impossible to focus clearly on anything but the chromosome surface, which therefore looks like a series of disks or bands (Fig. 4, *a*). In the model of a single helical wire (Fig. 4), the distance between gyres is indicated to be either compressed (as at *a* or *e*) or in varying degrees of looseness (as at *b–d*). The loose areas show structure comparable with Figure 1 but fail to show the possible breakage of coils (as at *c*, *d*, or *e* in Fig. 1). A heavy band such as that at *e* in Figure 1 might arise as a tight concentration of coils as shown at *e* in Figure 4. However, there is another possible explanation to account for the apparent discontinuity between some bands, besides breakage, namely, the stretching of the strands so that they are below the

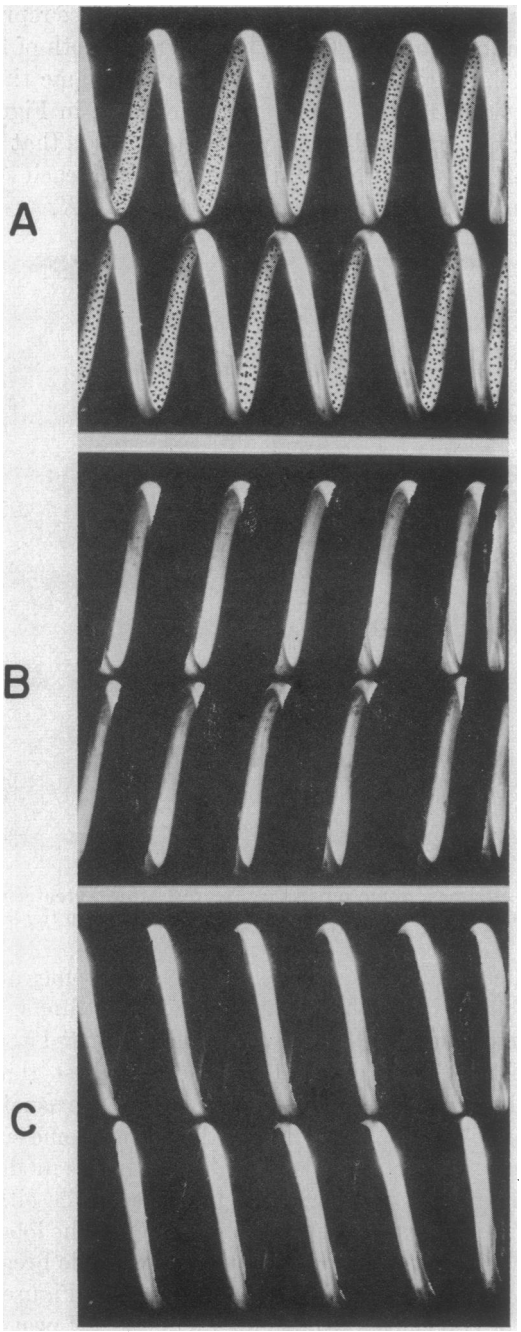

FIG. 3.—Metal models of helices arranged to show the appearance of a double helix (*A*), the same with the upper part of each coil blacked out (*B*), and the same with the lower part blacked out (*C*).

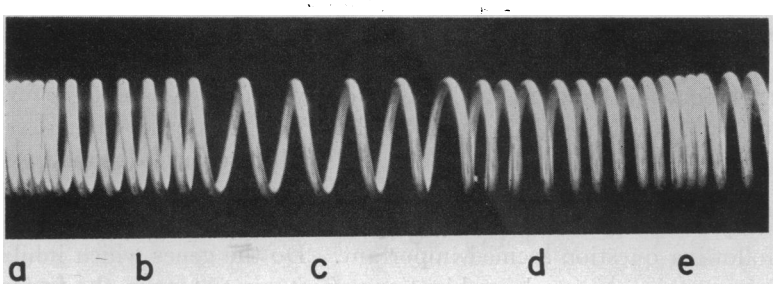

FIG. 4.—Model of a single helix with variable distances between the coils to illustrate the possible similarity to the giant chromosomes.

resolving power of the microscope and are thus invisible. This point will be brought out in a later paper that will consider the multiple-strand nature of the chromosome and of their hierarchy of helices.

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*HETEROCARYOSIS AND PROTOPLASMIC INCOMPATIBILITY IN NEUROSPORA CRASSA**

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Introduction.—The physiological basis of incompatibility between organisms is a subject of considerable interest. Although incompatibility reactions are known in less complex forms, they have been studied most intensively in the higher organisms—for example, the researches concerned with outbreeding in flowering plants and blood types in animals. Among the micro-organisms, certain incompatibilities described in the past are related to sex, e.g., the mating-type factors of some Protozoa and fungi. In contrast, incompatibilities recently described in strains of *Neurospora crassa* appear to be related primarily to the vegetative stages. This